The Preclinical Characterization of TST005, a Bi-functional Anti-PD-L1 and TGF-β Trap Fusion Protein

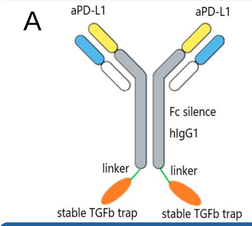
Hongjun Li[1], Chunming Wang[1], Huanhuan Guo[1], Xinlai Yao[1], Weiwei Sun[1], Di Sun[1], Yupeng Zhu[1], Xiangyu Xu[1], Chengwei Jiang[1], Shenjie Zhang[1], Xiwen Wu[1], Yingdi Luo[2], Yadong Dai[2], Lisa Zheng[1], Fan Zhang[1], Fei Teng[1] Xueming Qian[1], Yi Gu[1].

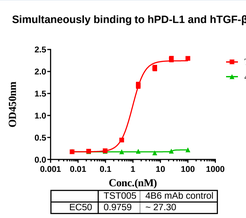
[1]Mabspace Biosciences (Suzhou) Co., Limited, Suzhou, China; [2]HJB (Hangzhou) Co, Limited, Hangzhou, China

ABSTRACT

Treatment with immune checkpoint inhibitors, including anti-PD(L)-1 antibodies, have demonstrated durable responses in patients with PD-L1 expressing tumors. However, patients with de novo or acquired resistance to checkpoint inhibitors have failed the treatment despite PD-L1 expression. The evidence from a number of studies showed that transforming growth factor β (TGF-β) signaling in the tumor microenvironment is associated with a poor prognosis, and inhibition of TGF-β signaling can induce tumor T cell infiltration/activation and potentiate tumor response to immune checkpoint therapy. TST005 is a bi-functional fusion protein composed of the truncated extracellular domain of the TGF-βRII receptor (a TGF-β trap) fused to a humanized anti-PD-L1 IgG1 antibody (4B6 mAb control) with ablated Fc immune effector function. TST005 bound to human/monkey PD-L1 with high affinity and blocked the PD-1/PD-L1 interaction potently in a cell-based reporter assay. Similarly, TST005 also bound to TGF-β1, 2 and 3 isoforms with high affinity and inhibited the activation of TGF-β receptor mediated signaling. In a superantigen stimulation assay with human peripheral blood mononuclear cells (PBMCs), TST005 enhanced T cell activation significantly as measured by interferon-γ (IFN-γ) production, as compared to TGF-β trap or 4B6 mAb control alone. In contrast, TST005 did not exert any cytokine release effect on human naïve PBMCs *in vitro*. TST005 showed a linear dose-proportional PK profile with a single IV infusion in mice, and a negative correlation between plasma TST005 concentration and its pharmacodynamic marker TGF-β1 level was established. Furthermore, in the MC38/hPD-L1 xenograft mouse model, compared with TGF-β trap or 4B6 mAb control, TST005 increased the infiltration of activated CD8+ T cells into the tumor, and it resulted in tumor regression starting from 3mg/kg. Of particular interest, in the EMT6/hPD-L1 xenograft model which responds only moderately to PD-L1 inhibitors, TST005 induced significant tumor growth inhibition and regression starting from 10mg/kg. In conclusion, we have demonstrated that TST005 has enhanced immunomodulatory properties and can induce potent antitumor activity in preclinical tumor models that are not sensitive to PD-1/PD-L1 monotherapy. These results provide the rationale for further clinical evaluation of TST005 in patients with advanced solid tumors and less optimal response to first generation PD(L)-1 based immunotherapy.

About TST005

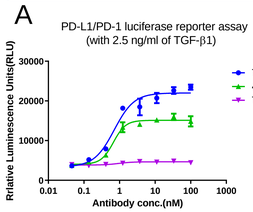




	TST005	4B6 mAb control
EC50	0.9759	~ 27.30

Figure 1. (A) Structure of TST005 bi-functional fusion protein (B). TST005 simultaneously binds to human PD-L1 and TGF-β1 by ELISA. TGF-β1-coated plates were incubated with serial dilutions of TST005 or 4B6 mAb control, followed by adding biotinylated PD-L1.

Cell-based Activity of TST005



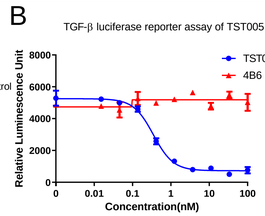


Figure 2. Functional activity of TST005 in cell-based bioassays (A) PD-L1/PD-1 luciferase reporter assay of TST005 and 4B6 mAb control (B) TGF-β luciferase reporter assay of TST005 and 4B6 mAb control

Effect of TST005 on Tuberculin (TB) Stimulated human PBMC

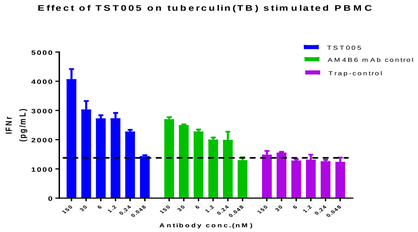
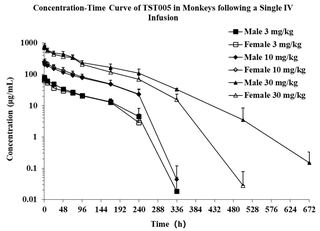
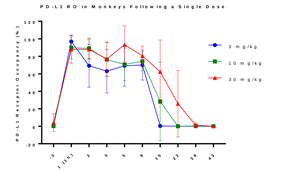


Figure 3. TST005 activates T lymphocytes stimulated with tuberculin (TB) to release IFN-γ in a dose-dependent manner. The serially diluted antibody was incubated with activated human PBMC stimulated by tuberculin (TB) for 3 days. IFN-γ in the supernatant from human PBMC was measured by ELISA.

PK-PD Relationship of TST005 in non-Human Primates





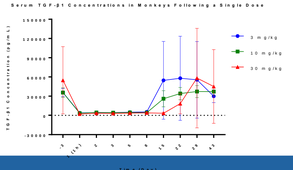
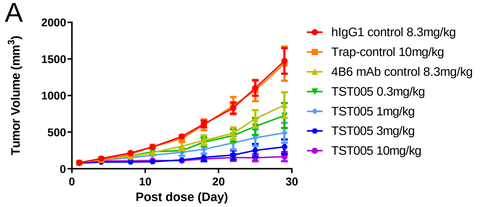
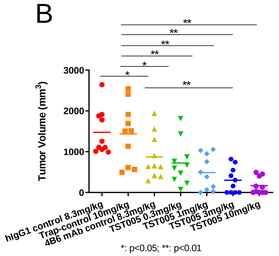


Figure 4. Serum TST005 concentration-time curve and changes of PD markers (PD-L1 receptor occupancy, serum TGF-β1 and TGF-β2 level) in Cynomolgus monkeys after a single dose of TST005 via IV infusion. Remarkable occupancy of PD-L1 and depletion of serum TGF-β1 were observed after TST005 administration, the changes of these PD markers were correlated with the serum TST005 concentrations and dose levels.

Efficacy of TST005 in MC38/hPD-L1 Tumor Model





*: p<0.05; **: p<0.01

Figure 5. TST005 inhibited MC38/hPD-L1 tumor growth（mean ± S.E.M, n=10）. A. Tumor growth curve B. Individual tumor volume on Day 29.

TST005 Increased TILs in MC38/hPDL1 Tumor Model

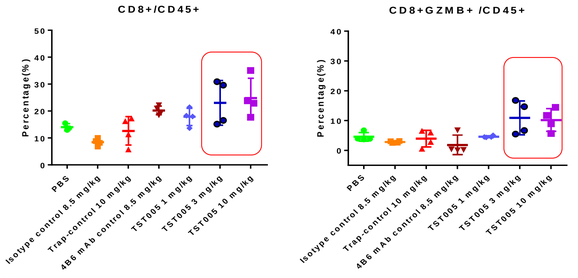
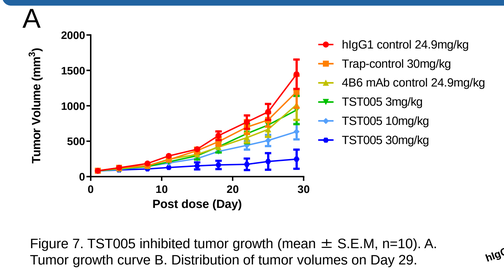
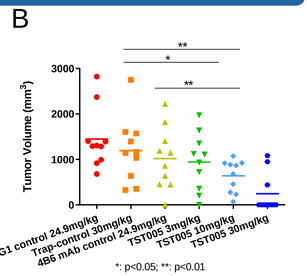


Figure 6. Tumor infiltrating lymphocytes (TILs) analysis in MC38/hPD-L1 tumor-bearing mice treated with TST005 via IV injection twice weekly for two weeks (Mean ± S.E.M, n=4)

Efficacy of TST005 in EMT6/hPD-L1 Tumor Model





*: p<0.05; **: p<0.01

Figure 7. TST005 inhibited tumor growth (mean ± S.E.M, n=10). A. Tumor growth curve B. Distribution of tumor volumes on Day 29.

CONCLUSIONS

- TST005 is a bi-functional fusion protein designed to simultaneously target two immune suppressive pathways, blocking PD-L1 and reducing TGF-β signaling, and it demonstrated superior anti-tumor activity in pre-clinical studies as compared to anti-PD-L1 or TGF-β-trap control alone.
- Fc engineering eliminates TST005's binding affinity to FcγRIIIA and C1q, thereby ADCC or CDC activity of TST005 is abolished.
- According to the PK-PD study in cynomolgus monkeys, the effects of TST005 on PD-L1 receptor occupancy, and the concentration changes of serum TGF-β1 were correlated with serum TST005 concentrations over the dose range of 3-30 mg/kg following a single IV administration.
- The first-in-human study is expected 2021.

REFERENCES

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Corresponding author: hongjun.li@transcenta.com